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Voluntary exercise decreases ethanol preference and consumption in C57BL/6 adolescent mice: Sex differences and hippocampal BDNF expression

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HIGHLIGHTS

- We studied the effects of wheel running on ethanol consumption in adolescent mice.
- We identified sex-differences in the effects of exercise on alcohol drinking.
- The amount of distance traveled affects ethanol consumption in female mice.
- Hippocampal BDNF expression increased after voluntary exercise.

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ABSTRACT

Adolescence is a period of high vulnerability for alcohol use and abuse. Early alcohol use has been shown to increase the risk for alcohol-related problems later in life; therefore effective preventive treatments targeted toward adolescents would be very valuable. Many epidemiological and longitudinal studies in humans have revealed the beneficial effects of exercise for prevention and treatment of alcohol addiction. Pre-clinical studies have demonstrated that access to a running wheel leads to decreased voluntary alcohol consumption in adult mice, hamsters, and rats. However, age and sex may also influence the effects of exercise on alcohol use. Herein, we studied male and female C57BL/6 adolescent mice using a 24-hour two-bottle choice paradigm to evaluate 21 days of concurrent voluntary exercise on alcohol consumption and preference. Given previously known effects of exercise in increasing the expression of brain-derived neurotrophic factor (BDNF) in the hippocampus and its role in regulating the reward system, BDNF mRNA and protein levels were measured at the end of the behavioral experiment. Our results demonstrate sex differences in the efficacy of voluntary exercise and its effects on decreasing alcohol consumption and preference. We also report increased BDNF expression after 21 days of voluntary exercise in both male and female mice. Interestingly, the distance traveled played an important role in alcohol consumption and preference in female mice but not in male mice. Overall, this study demonstrates sex differences in the effects of voluntary exercise on alcohol consumption in adolescent mice and points out the importance of distance traveled as a limiting factor to the beneficial effects of wheel running in female mice.

1. Introduction

It is well known that drinking alcohol beverages is a common feature of social gatherings due to its effects on improving mood and increasing self-confidence and sociability. However, the harmful use of alcohol leads to the death of 2.5 million people worldwide in a year (http://www.who.int/en/). The risk for alcohol use and abuse among adolescents may be particularly high since this is a period during which social contexts exert powerful influences. Moreover, behavioral characteristics common among adolescents, such as enhanced novelty seeking, sensation seeking and reward seeking behavior along with poor judgment and lack of impulsive control, are likely to contribute to increased risk for alcohol use (reviewed in [1–4]). Importantly, earlier initiation of alcohol use (prior to age 15) has been associated with increased risk for alcohol-related problems later in life [5]. Therefore, it is necessary to find preventive treatments targeted toward adolescents.

Physical activity has been proposed to be a potential non-pharmacological treatment for alcohol addiction. Epidemiological data obtained from adolescents show that, when engaged in regular exercise,

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teens are less likely to consume alcohol [6–13]. Longitudinal studies also show that persistently active individuals report lower levels of alcohol use, during both adolescence and early adulthood [14], as well as fewer alcohol-related problems, particularly in women [15]. However, exercise does not always prevent the use of alcohol and, in some specific situations where high level of physical activity is required, is associated with increased use of alcohol. For example, female athletes involved in mixed-gender sports and male athletes involved in male-dominated sports show higher rates of alcohol use [16–18], suggesting that gender and both the type and intensity of exercise may also influence alcohol use.

Studies from animal models have shown variability in the effects of exercise on voluntary alcohol consumption, depending on the behavioral model and gender examined (reviewed in [19]). The majority of these studies have been performed using adult animals, and little is known about the effects of wheel running on alcohol consumption during adolescence. It is important to characterize these effects, because adolescence is not only a period during which experimentation with alcohol begins, but also a period of brain maturation, making it highly vulnerable to environmental changes. Brain-derived neurotrophic factor (BDNF) has been shown to play a significant role in neuronal development and synaptic plasticity whose levels peak during adolescence with the highest level of BDNF detected in the hippocampus (reviewed in [20]). Furthermore, BDNF has been suggested to be a key molecule in regulating hippocampal functions, such as its role in the reward system [21] or the HPA axis activity [22]. Importantly, differences in expression of genes involved in stress-related pathways have been demonstrated in hippocampi from brains of deceased alcoholics [23]. On the other hand, exercise is rewarding, has been shown to increase BDNF levels in the hippocampus (reviewed in [24]) and to alter the HPA axis responsiveness [25]. Collectively, these studies justify careful examination of this neurotrophin in the hippocampus to determine its possible mechanistic role on the effects of exercise on alcohol consumption during adolescence.

Thus, the first goal of this study was to examine the effects of 21 days of concurrent voluntary wheel running on ethanol consumption and preference in adolescent C57BL/6 mice. Our results demonstrate sex differences in the effects of exercise on alcohol drinking. In a second goal, we measured mRNA and protein levels of BDNF expression in an attempt to understand some of the underlying neuronal mechanisms contributing to these behaviors. As seen in previous animal studies [26], we observed differences in BDNF expression after ethanol and exercise exposure, suggesting that BDNF may be involved in responses to exercise and alcohol, which merits further study.

2. Material and methods

2.1. Animals

Male and female adolescent (21–25 days) C57BL/6Ibg mice were obtained from breeder pairs at the Institute for Behavioral Genetics at the University of Colorado, Boulder (Boulder, Colorado). The animals were housed in standard mouse cages with ad libitum water and standard rodent chow (Harlan Laboratories, Indianapolis, Indiana) until the start of the experiment. The lighting in the animal colony was maintained on a 12-hour light/dark cycle with lights on at 7:00 AM. Room temperature was maintained at 23 ± 1.5 °C.

This study was conducted with approval from the Institutional Animal Care and Use Committee at the University of Colorado, Boulder (Boulder, Colorado) following guidelines established by the Office of Laboratory Animal Welfare (OLAW). All possible measures were taken to minimize discomfort.

2.2. Behavioral paradigm

Male (n = 15–18/condition) and female (n = 14–18/condition) C57BL/6Ibg mice were weaned at 21–25 days old and placed in individual cages (332 × 150 × 130 mm), with or without running wheels (Coulbourn Instruments, PA, USA) and two cylinder tubes (25 ml) of water. After 2 days of acclimation, mice were tested using previously established 24-hour two-bottle choice paradigm conditions that lead to differences in voluntary ethanol consumption [27]. The four conditions included cages with 1) water only (Sedentary-Water), 2) water and 10% ethanol two-bottle choice (Sedentary-Ethanol), 3) water only with a running wheel (Exercised-Water), and 4) water and 10% ethanol two-bottle choice with a running wheel (Exercised-Ethanol). The mice housed with a running wheel had 24-hour access to the wheel. The protocol lasted 21 days. The side of the tubes was alternated every two days in order to account for any side preference. Individual consumption of water and ethanol (if applicable) were recorded daily. Distance and time were counted and recorded daily using a Cat Eye Mity 8 cyclocomputer (Cateye America, CO, USA). On day 21, mice were sacrificed by cervical dislocation, and brains were removed for subsequent BDNF expression analysis. The hippocampi of a group of mouse brains were dissected and stored at −70 °C until the time to perform the below BDNF ImmunoAssay.

2.3. BDNF immunoassay

BDNF protein was measured in the hippocampus of mice at the end of the behavioral experiment using a Promega BDNF Emax ImmunoAssay System (Promega Co., WI, USA) according to the manufacturer's protocol.

2.3.1. Extraction of protein from mouse brain hippocampi

Male (n = 5–6/condition) and female (n = 5–6/condition) hippocampi were removed from the freezer, and then weighed. Two milliliters of Promega lysis buffer (137 mM NaCl, 20 mM Tris–HCl (pH 8.0), 1% NP40, 10% Glycerol) were added to each 100 mg of tissue along with a protease inhibitor cocktail (Thermo Fisher Scientific, IL, USA). Samples were homogenized with a syringe and then sonicated with a Branson 1210 sonication water bath (EquipNet, MA, USA). The samples then were centrifuged at 16,000 × *g* for 30 min at 4 °C. The resulting supernatant was pipetted into a new tube and stored at −70 °C until use.

2.3.2. BDNF protein quantification

Each well of a 96-well polystyrene plate, included in the Promega BDNF Emax ImmunoAssay kit, was coated overnight at 4 °C, with anti-BDNF monoclonal antibody (mAb), by mixing 10 μl of mAb with 9.99 ml of carbonate coating buffer (25 mM sodium bicarbonate and 25 mM sodium carbonate, pH 9.7). Unabsorbed mAb was removed and plates were washed once with TBST wash buffer (20 mM Tris–HCl (pH 7.6), 150 mM NaCl and 0.05% (v/v) Tween 20). The plates were blocked using 200 μl Promega 1X Block & Sample buffer and incubated for 1 h at room temperature. In the meantime, tissue extracts were removed from the freezer and thawed on ice. After 1 h of incubation, plates were emptied and washed once in TBST. One hundred microliters of each sample or standard (1000, 750, 500, 300, 200, 100, 0 pg/ml) were added in triplicate to the plates and then incubated with shaking for 2 h at room temperature. Plates were emptied and washed five times using TBST. One hundred microliters of 1:500 Anti-human BDNF polyclonal antibody (pAb), diluted in 1X Block & Sample buffer, was added to each well plate. Plates then were incubated again with shaking for 2 h at room temperature. Plates were emptied and washed five times using TBST. One hundred microliters of 1:200 Anti-IgY horseradish peroxidase conjugate, diluted in 1X Block & Sample buffer, was added to each well plate. Plates then were incubated again with shaking for 1 h at room temperature. Plates were emptied and washed again five times using TBST. Finally, plates were developed using 100 μl Promega TMB One Solution and the reaction was stopped using 100 μl 1 N HCl. Absorbance was measured at 450 nm. BDNF levels are reported in pg/mg total protein ± SEM.